

1

METHODS FOR VIRAL INACTIVATION AND OTHER ADVENTITIOUS AGENTS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Patent Application No. 61/662,349, filed Jun. 20, 2012, the disclosure of which is incorporated by reference herein in its entirety.

FIELD OF INVENTION

The invention provides for methods of viral inactivation using high temperature short time (HTST) treatment and adjustment of various parameters such that generation and depositions of precipitate is reduced and/or minimized.

BACKGROUND OF THE INVENTION

Viruses are potential contaminants in drug manufacturing processes, particularly in cases where biologic drugs are derived from mammalian cell cultures. A source of viral contaminants can be the media used for cell culture or the cell lines producing the biologics of interest. Current approaches to prevent viral contamination of biologic drugs during the manufacturing process includes high temperature short time (HTST) cell media treatment for the inactivation of viruses that may be introduced into cell culture media by raw materials and is amplified during the culturing process (Schleh, M. et al. 2009. *Biotechnol. Prog.* 25(3):854-860 and Kiss, R. 2011. *PDA J Pharm Sci and Tech.* 65:715-729). It has been reported that temperatures in excess of about 85° C. are needed for HTST to be an effective virus inactivation method, with temperatures in excess of about 95° C. needed to inactivate parvovirus, a common cell culture viral contaminant that has been documented as occurring in cell culture processes, and which is resistant to many chemical and physical inactivating agents (Schleh et al.).

Although HTST treatment has proven to be highly effective in the inactivation of viruses, precipitation or formation of precipitates can occur in various cell culture media when subjected to this treatment. This precipitation leads to an accumulation of residue on the surfaces within the HTST system and can contribute to fouling of the equipment such that it can no longer heat up the media to the target temperature for proper inactivation of viral contaminants. Additionally, such precipitation can also foul the filters typically used downstream of the HTST system for the final processing to remove microorganisms, such as bacteria, from the medium. Such filter fouling can lead to inability to complete the medium processing step prior to the cell culture process. In some instances the precipitate may also impact the performance of the cell culture media and prevent efficient production of biologic drugs from the cultured cell lines. To prevent precipitation, the temperature can be lowered but successful viral inactivation may be negatively affected. Furthermore, precipitate formation during HTST cell media treatment can result in frequent cleaning or repair of equipment used for HTST treatment during the manufacturing process which contributes significantly to the cost of processing. Therefore, there is a need for methods to prevent precipitate formation during HTST treatment without adversely affecting the efficacy of this treatment in the removal or inactivation of viral contaminants.

The invention described herein addresses these needs by providing methods to effectively inactivate viral contaminants

2

in cell culture media using HTST treatment with adjusted processing parameters that results in the reduction or prevention of precipitate formation.

All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

BRIEF SUMMARY OF THE INVENTION

The invention provides for methods, processes, systems and compositions for inactivating viral contamination and/or other contaminants in cell culture media by using high temperature short time (HTST) treatment in combination with adjustments of various parameters, such as pH and/or calcium and/or phosphate concentration in the media. Furthermore, methods, processes, systems and compositions reducing the fouling of equipment and filters used for HTST treatment are provided as well.

Accordingly, in one aspect, the invention provides for methods for inactivating virus or adventitious agents in cell culture media while the media maintains suitability for cell culture, said method comprising (a) subjecting the cell culture media to high temperature short time (HTST) treatment; and (b) adjusting one or more parameters selected from the group consisting of pH, calcium level and phosphate level.

In other aspects, the invention provides for methods for inactivating virus in cell culture media comprising subjecting the cell culture media to high temperature short time (HTST) treatment wherein the media has a pH of between about pH 5.0 to about pH 6.9 during HTST treatment. In another aspect, the invention provides for methods for inactivating virus in cell culture media comprising subjecting the cell culture media to high temperature short time (HTST) treatment wherein the media has a pH of between about pH 5.0 to about pH 7.2 during HTST treatment. In some embodiments, the media has a pH of between about pH 5.3 to about pH 6.3 during HTST treatment. In other embodiments, the media has a pH of about pH 6.0 during HTST treatment. In any of the embodiments, the HTST treatment comprises raising the temperature of the media to at least about 85 degrees Celsius for a sufficient amount of time to inactivate the virus or potential virus in the media. In some embodiments, the temperature of the media is raised to at least about 93 degrees Celsius for a sufficient amount of time to inactivate the virus or potential virus in the media. In some embodiments, the temperature of the media is raised to at least about 95, 97, 99, 101 or 103 degrees Celsius for a sufficient amount of time to inactivate the virus or potential virus in the media. In some embodiments, the pH of the media is lowered to between about pH 5.0 to about pH 6.9 during HTST treatment prior to polypeptide production phase. In some embodiments, the pH of the media is then brought to between about 6.9-7.2 for the polypeptide production phase.

In another aspect, the invention provides methods for inactivating virus in cell culture media comprising limiting the total amount of phosphate and calcium in the media to less than about 10 mM during HTST treatment. In some embodiments, the total phosphate and calcium concentration in the media is limited to less than about 9, 8, 7, 6, 5, 4, 3, 2, or 1 mM during HTST treatment. In some embodiments, the total amount of phosphate and calcium in the media is limited to less than about 10 mM during HTST treatment prior to polypeptide production phase. In some embodiments, the total amount of phosphate and calcium in the